Donor Polymorphisms in Genes Related to B-Cell Biology Associated With Antibody-Mediated Rejection After Heart Transplantation

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Background: Heart transplantation (HT) is a well-established lifesaving treatment for endstage cardiac failure. Antibody-mediated rejection (AMR) represents one of the main problems after HT because of its diagnostic complexity and the poor evidence for supporting treatments. Complement cascade and B-cells play a key role in AMR and contribute to graft damage. This study explored the importance of variants in genes related to complement pathway and B-cell biology in HT and AMR in donors and in donor-recipient pairs.

Methods and Results: Genetic variants in 112 genes (51 complement and 61 B-cell biology genes) were analyzed on next-generation sequencing in 28 donor-recipient pairs, 14 recipients with and 14 recipients without AMR. Statistical analysis was performed with SNPStats, R, and EPIDAT3.1. We identified one single nucleotide polymorphism (SNP) in donors in genes related to B-cell biology, *interleukin-4 receptor subunit α* (p.Ile75Val-IL4Ra), which correlated with the development of AMR. Moreover, in the analysis of recipient-donor genotype discrepancies, we identified another SNP, in this case in *adenosine deaminase* (ADA; p.Val178(p=)), which was related to B-cell biology, associated with the absence of AMR.

Conclusions: Donor polymorphisms and recipient-donor discrepancies in genes related to the biology of B-cells, could have an important role in the development of AMR. In contrast, no variants in donor or in donor-recipient pairs in complement pathways seem to have an impact on AMR.

Key Words: Antibody-mediated rejection; B-cell gene; Donor genotype; Heart transplantation

Heart transplantation (HT) is a well-established life-saving treatment for endstage cardiac failure.¹ Antibody-mediated rejection (AMR), however, represents one of the main problems for clinical management of HT because of its diagnostic complexity and poor evidence for supporting treatment.²

The molecular mechanism involved in AMR is still unknown, but an important role of the complement cascade and B cells has been established.^{2,3} On the one hand, complement activation triggers a strong inflammatory response, and also generates tissue-bound and soluble fragments related to AMR.⁴ And on the other hand, B cells

play an important role in the immune response by producing antibodies, presenting antigens to T cells, and secreting cytokines.^{5,6} In both pathways, there are more than 120 genes encoding proteins (isotypes and subunits; **Figure 1**) involved in complement activation, immunoglobulin gene rearrangement, maturation, activation and differentiation of B cells.^{7,8}

There have been many attempts to associate solid organ allograft outcomes with specific genetic variants,⁹ but most studies have focused on recipient genotypes. Our group recently found that polymorphisms in recipients in complement¹⁰ and in B-cell pathways (Núñez et al, unpublished

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Figure 1. Complement pathway and B-cell development and activation. Summary of the main genes implicated in both pathways. ADA, adenosine deaminase.

data, 2018) are associated with AMR. In recent years there has been increasing interest in donor genotype associated with allograft outcome.^{11–15} With regard to complement and B-cell pathways, there are, at least, 22 studies (Table 1) on the association between donor or donor-recipient pair genotype and different solid organ transplant outcomes.^{14,16–36} Sixteen of the 22 studies noted an association between single nucleotide polymorphism (SNP) and transplant outcome, indicating the importance of genetics in this field. Most of the studies, however, analyzed the association between one or two genes and different outcomes, in which the complement and B-cell role is not always clear. Surprisingly, despite the importance of complement pathway or B-cell biology in AMR, there have been no studies on the association between donor variants in these pathways and AMR. Moreover, it is important to note that none of the complement studies was performed in the field of HT, and only five studies related to B-cell biology were conducted in the field of HT.^{27,29,31–33} Given these findings, the aim of this study was therefore to examine the importance of the genes related to complement pathway and B-cell biology in HT and AMR in donors and in donor-recipient pairs.

Methods

Patients

A total of 14 patients with and 14 patients without AMR

(controls) after HT and their corresponding donors were selected for the study (total subjects, n=56). The diagnostic criteria for AMR differed according to whether the transplant date was before or after 2013. AMR in patients with transplantation before 2013 (n=7) was defined according to the following criteria: (1) allograft dysfunction (left ventricular ejection fraction [LVEF], <30% and/or heart failure); (2) no evidence of other causes of allograft dysfunction (acute cellular rejection or cardiac allograft vasculopathy); (3) evidence of complement activation on endomyocardial biopsy (EMB; C4d and/or C3d staining); and/or (4) favorable response to therapy addressing AMR (including plasmapheresis, rituximab, steroids boluses, i.v. immunoglobulin etc.). AMR in patients with transplantation after 2013 (n=7) was classified according to International Society for Heart and Lung Transplantation (ISHLT) categories,^{37,38} and the inclusion criterion was at least 1 positive EMB (pAMR1 or higher). Controls did not have any distinguishing signs of AMR (pAMR0), or allograft dysfunction. All samples were from the “Colección de Muestras Para la Investigación de Insuficiencia Cardíaca Avanzada y Trasplante Cardíaco” registered in the Institute of Health Carlos III (C_0000419, 2012/348). The study was performed in accordance with the Declaration of Helsinki and was approved by the ethics committee of “Investigación de Galicia” (ref: 2014/012).

Table 1. Previous Studies on Donor or Recipient-Donor Gene Variants Associated With Allograft Outcome

	Organ transplanted	D and R	Gene studied (polymorphism)	No. patients	Outcome	Findings
COMPLEMENT PATHWAY						
Brown et al (2006) ²⁶	Kidney	D-R pairs	C3 (rs2230199)	501	Graft outcome	No association
Varagunam et al (2009) ²⁵	Kidney	D-R pairs	C3 (rs2230199)	1,147	Graft outcome	D-R discrepancies → worst prognosis
Cervera et al (2009) ¹⁴	Liver	D	<i>MBL2</i> (promoter and rs5030737, rs1800450, and rs1800451)	95	Infections, AR, and graft survival	D with rs5030737, rs1800450, and rs1800451 → worst prognosis
Dhillon et al (2010) ²⁴	Liver	D-R pairs	C3 (rs2230199)	296	Graft outcome	No association
Jeong et al (2011) ²²	Kidney	D-R pairs	C5 (rs2159776, rs17611, rs25681 and rs2241004 (GGCG haplotype)) and <i>C5aR</i> (rs10404456)	191	Graft outcome	↓ Renal function in GGCG in R and D. No association in <i>C5aR</i>
Wahrmann et al (2011) ²³	Kidney	D-R pairs	C4 (CNVs)	1,969	Graft survival	No association
Bazayr et al (2012) ²⁰	Kidney	D-R pairs	C3 (rs2230199)	100	AR	No association
Damman et al (2012) ²¹	Kidney	D-R pairs	C3 (rs2230199)	1,265	PNF, AR, DGF, PS and graft outcome	D polymorphism associated with PNF
Budding et al (2016) ¹⁷	Lung	D	<i>CD59</i> (promoter)	137	Graft survival	D polymorphism: impaired long-term survival and ↑ incidence of BOS
Ermini et al (2016) ¹⁶	Kidney	D-R pairs	47 genes (505 tagged SNP)	Two cohorts of 650 and 520 patients	Graft outcome	No association
B-CELL BIOLOGY						
Sankaran et al (1999) ³⁶	Kidney	D and R	<i>TNFA</i> (promoter) and <i>IL10</i> (promoter)	115	RES and RS	R <i>TNFA</i> and <i>IL10</i> polymorphisms are determinant in RES and RS
Awad et al (2001) ³²	Pediatric heart	D and R	<i>TNFA</i> (promoter), <i>IL10</i> (promoter), <i>IL6</i> (promoter), <i>TGFB1</i> (rs1800470 and rs1800471) and <i>IFNY</i> (rs2430561)	93 R and 29 D	AR	R polymorphisms associated with AR
Holweg et al (2001) ³³	Heart	D and R	<i>TGFB1</i> (rs1800470 and rs1800471)	252 R and 213 D	Chronic allograft failure	R polymorphisms associated with chronic allograft failure
Poole et al (2001) ³⁴	Kidney	D-R pairs	<i>IL10</i> (promoter), and <i>IL4</i> (promoter)	120	AR and graft outcome	D and R <i>IL4</i> and <i>IL10</i> genotypes influence renal transplantation outcome and AR
Marshall et al (2001) ³⁵	Kidney	D and R and D-R pairs	<i>IL6</i> (promoter)	145 D, 209 R and 126 pairs	AR	D <i>IL6</i> genotype → major genetic risk of AR
Bijlsma et al (2002) ³¹	Heart	D and R	<i>IL4</i> (Promoter)	167	AR	D genotype ↓ AR
Densem et al (2004) ²⁹	Heart	D and R	<i>TGFB1</i> (rs18041006 and rs1982073)	147 R and 134 D	CV	R rs18041006 ↑ CV
Hoffmann et al (2004) ³⁰	Kidney	D	<i>IL2</i> , <i>IL6</i> , <i>IL10</i> , <i>TNFA</i> , <i>TGFB</i> , <i>IFNY</i> , <i>CCR2</i> , <i>CCR5</i> (polymorphism related with AR)	68	AR	D polymorphisms → influence R immune response
Karabon et al (2005) ²⁸	Hematopoietic stem cell	D and R	<i>IL6</i> and <i>IL10</i> (promoter)	93 R and 74 D	aGVHD	D polymorphism <i>IL10</i> and <i>IL6</i> → effect in the risk of aGVHD
Fildes et al (2005) ²⁷	Heart	D and R	<i>CCR5</i> (rs333 (CCR5Δ32))	178	Graft outcome	D genotype → mortality
Biggins et al (2013) ¹⁸	Liver	R and D-R pairs	<i>IL28B</i> (rs12979860 and rs8099917) <i>DDX58</i> (rs10813831)	440 R and 225 D-R pairs	Severity of HCV recurrence	D genotype ↑ Risk HCV
Firpi et al (2013) ¹⁹	Liver	D-R pairs	<i>IL28B</i> (rs12979860)	135	HCV outcome	D and R genotype → better response to treatment

aGVHD, acute graft-vs.-host disease; AR, acute rejection; BOS, bronchiolitis obliterans syndrome; CV, coronary vasculopathy; D, donor; DGF, delayed graft function; HCV, hepatitis C virus; PNF, primary non-function; PS, patient survival; R, recipient; RES, steroid-resistant or responsive; RS, rejection severity.

Table 2. Baseline Subject Characteristics

Variable	AMR		Non-AMR	
	Recipients (n=14)	Donors (n=14)	Recipients (n=14)	Donors (n=14)
Age (years)	45.7±4.9	46.5±3.2	45.3±4.7	36.3±2.9
Male	78.6 (11)	64.3 (9)	78.6 (11)	71.4 (10)
Female	21.4 (3)	35.7 (5)	21.4 (3)	28.6 (4)
Primary heart disease				
Dilated cardiomyopathy	21.4 (3)		28.6 (4)	
Ischemic cardiomyopathy	42.9 (6)		35.7 (5)	
Valvular cardiomyopathy	7.1 (1)		7.1 (1)	
Others	28.6 (4)		28.6 (4)	
Time since transplant (years)				
<2	28.6 (4)		7.1 (1)	
2–5	28.6 (4)		14.3 (2)	
≥6	42.9 (6)		78.6 (11)	
Time to AMR diagnosis after transplant (years)	2.4±0.6			
CMV serology status				
D+/R+	64.3 (9)		64.3 (9)	
D–/R–	0		0	
D+/R–	21.4 (3)		28.6 (4)	
D–/R+	14.3 (2)		7.1 (1)	
Immunosuppression				
Cyclosporine	57.1 (8)		28.6 (4)	
Plasmapheresis	51.1 (8)		0	
Steroids	100 (14)		100 (14)	
Mycophenolate mofetil	85.7 (12)		92.9 (13)	
Tacrolimus	78.6 (11)		50.0 (7)	
Everolimus	35.7 (5)		42.9 (6)	
Basiliximab	92.6 (13)		92.6 (13)	
Daclizumab	0		7.1 (1)	
Rituximab	51.1 (8)		0	
Thymoglobulin	7.1 (1)		0	

Data given as mean ± SD or % (n). AMR, antibody-mediated rejection; CMV, cytomegalovirus; D, donor; R, recipient.

Targeted Next-Generation Sequencing Genetic Analysis

Genomic DNA from the patients with and without AMR and their corresponding donors (n=56) was extracted from clots and blood samples using QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany) as previously described.¹⁰ All samples were included in the next-generation sequencing (NGS) study using the TruSight One panel according to manufacturer instructions (Illumina, San Diego, CA, USA). The TruSight One is a commercial NGS panel for the targeted genomic enrichment of 4,813 genes including 51 genes related to the complement pathway and 61 genes related to the biology of B cells (Table S1). The samples were paired-end sequenced on a NextSeq500 platform (Illumina) and mapped to the human genome reference sequence (GRCh37, hg19).

Databases and *In Silico* Tools

The potential effect of SNP associated with the presence of AMR was predicted using *in silico* tools as previously described.¹⁰ Moreover, the minor allele frequency (MAF) of the SNP described was checked on two different databases: Single Nucleotide Polymorphism Database (dbSNP) and/or Exome Aggregation Consortium (ExAC).

Variant Localization Topological placement of the mutations was done using the Swissprot database (<http://ca.expasy.org/uniprot/>).

The Uniprot database provides generally accepted residue ranges corresponding to each domain region and specialized subregion.

Prediction of Damaging Amino Acid Substitution Five online tools were used to predict the pathogenicity of the missense variants: SIFT (http://sift.jcvi.org/www/SIFT_seq_submit2.html), Polyphen-2 (<http://genetics.bwh.harvard.edu/pph2/>), PhDSNP (<http://snps.biofold.org/phd-snp/phd-snp.html>), SNAP2 (<https://www.rostlab.org/services/snap/>), and MutationTaster (<http://www.mutationtaster.org>).

Prediction of Exonic Splicing Enhancer Variants The effect of synonymous variants in the splice-enhancing sequences was evaluated using HSF (<http://www.umd.be/HSF/>).

Quality of NGS Sequencing

The mean coverage of all the genes related to the complement pathway or the biology of B cells was as high as 83.7±2.5-fold. Direct sequencing of 24 different amplicons (Table S1), containing at least one variant on NGS each, was performed to evaluate sensitivity [TP/(TP+FN)], specificity [TN/(TN+FP)], and accuracy [(TP+TN)/(TP+FP+FN+TN)] of the NGS technique, where TP means true positive, TN means true negative, FP means false positive, and FN means false negative. After analyzing all

Table 3. Presence of Variant p.Ile75Val (rs1805010) in IL4R vs. AMR Status

rs1805010 IL4R p.Ile75Val / Model	Genotype	Non-AMR n (%)	AMR n (%)	OR (95% CI)	P-value	AIC	BIC
SNPstats							
Codominant	A/A	10 (71.4)	2 (15.4)	1.00	0.0073	33.6	37.5
	A/G	4 (28.6)	10 (76.9)	12.50 (1.85–84.44)			
	G/G	0 (0)	1 (7.7)	NA (0.00–NA)			
Dominant	A/A	10 (71.4)	2 (15.4)	1.00	0.0024	32.2	34.8
	A/G-G/G	4 (28.6)	11 (84.6)	13.75 (2.05–92.04)			
Recessive	A/A-A/G	14 (100)	12 (92.3)	1.00	0.22	39.9	42.5
	G/G	0 (0)	1 (7.7)	NA (0.00–NA)			
Overdominant	A/A-G/G	10 (71.4)	3 (23.1)	1.00	0.01	34.8	37.4
	A/G	4 (28.6)	10 (76.9)	8.33 (1.47–47.23)			
Log-additive	–	–	–	12.99 (1.99–84.58)	0.0018	31.6	34.2
R (F(x) association)							
Codominant	A/A	10 (71.4)	2 (15.4)	1.00	0.0063	33.6	
	A/G	4 (28.6)	10 (76.9)	12.50 (1.85–84.44)			
	G/G	0 (0)	1 (7.7)	0.00			
Dominant	A/A	10 (71.4)	2 (15.4)	1.00	0.0024	32.2	
	A/G-G/G	4 (28.6)	11 (84.6)	13.75 (2.05–92.04)			
Recessive	A/A-A/G	14 (100)	12 (92.3)	1.00	0.4815	39.9	
	G/G	0 (0)	1 (7.7)	0.00			
Overdominant	A/A-G/G	10 (71.4)	3 (23.1)	1.00	0.0102	34.8	
	A/G	4 (28.6)	10 (76.9)	8.33 (1.47–47.23)			
Log-additive	–	–	–	12.99 (1.99–84.58)	0.0063	31.6	
Fisher's exact test (omnibus P-value)					0.0213		

AIC, Akaike information criterion; AMR, antibody-mediated rejection; BIC, Bayes information criterion; IL4R, interleukin 4 receptor.

the genes included in the study we obtained a sensitivity of 94.5%, a specificity of 100%, and an accuracy of 99.9%.

Statistical Analysis

For the association of donor genotype in genes related to complement and B-cell biology, multiple inheritance models (codominant, dominant, recessive, overdominant, and log-additive) were applied using SNPStats and the R association function. When these models showed a significant association, Fisher's exact test in R was used. The strength of association between SNP and AMR was estimated using OR, 95% CI, Akaike information criterion (AIC), Bayes information criterion (BIC) and P-value, using both SNPStats and R. Moreover, to identify recipient-donor pair genotype in genes related to the complement pathway and B-cell biology potentially associated with the risk of humoral rejection, Fisher's exact test in EPIDAT 3.1 was used. Due to the exploratory nature of these analyses, $P \leq 0.02$ was required in order to limit the risk of spurious associations.

Results

Baseline Clinical Characteristics

Baseline donor and recipients characteristics are listed in **Table 2**. In both groups of recipients, the most common transplant etiology was cardiomyopathy (71.4%). Using the pathologic AMR classification in patients after 2013 ($n=7$), based on a scale consisting of immunopathology and histological features,^{36,37} 4 patients were classified as pAMR(I+) and 3 patients as pAMR2. **Tables 2, S2** list

cytomegalovirus (CMV) serology status, immunosuppression regimen and AMR treatment.

Donor Genotype and AMR

Complement Genes A total of 51 genes related to the complement were analyzed in 28 donors (14 to patients with AMR and 14 to patients without AMR; **Table S1**). On sequencing of all coding and adjacent regions, from position –10 to +10, of the selected genes from the 28 HT donors, 257 SNP were identified (**Table S3**). After statistical analysis, none of the donor SNP in the complement pathway was associated with AMR.

Genes Related to B-Cell Biology A total of 61 genes related to B-cell biology were screened in 28 donors to 14 AMR patients and to 14 controls (**Table S1**). A total of 222 SNP were identified in the analyzed genes (**Table S3**), and one of these variants was significantly associated with AMR (codominant, $P=0.01$; dominant, $P=0.002$; overdominant, $P=0.01$; log-additive, $P=0.002$; **Table 3**). This polymorphism in *interleukin-4 receptor subunit α* (*IL-4R α*), an adenine to guanine substitution on the c.223 nucleotide that produces the substitution of the 75 lysine for valine (rs1805010; p.Ile75Val; **Figure 2**), was present more often in AMR donors than in control donors. We used *in silico* software to predict the effect on the function of the protein, and it was predicted to be tolerated by all the bioinformatics tools used (SIFT=tolerated (1), POLYPHEN=benign (0.001), PhDSNP=neutral, Mutation Taster=polymorphism (1,0), SNAP=neutral (87%), with the score of each *in silico* tool given in parentheses).

Recipient-Donor Genotype and AMR

The genotype of the most relevant genes in the complement pathway and in the biology of B cells was analyzed in 14 donor-recipient pairs in which the recipients developed AMR (n=28), and in 14 pairs in which the recipients did not (n=28). After the analysis of 112 genes, 662 SNP were identified (**Table S3**). The first analysis compared the total number of donor-recipient discrepancies in the 662 SNP between the AMR group and the control group. The mean number of discrepancies in the AMR group was 103.6 ± 3.3 whereas in the control group it was 108.7 ± 8.4 (P=NS).

The second analysis compared the frequency of each SNP in the donor-recipient pairs between the AMR and non-AMR groups. For this purpose, SNP with minor allele frequency <7%, meaning the presence of the alternative allele in <5 patients out of 56, were left out of further analysis (n=402, **Table S3**). Thus, we compared the distribution of genotype frequency in 260 SNP in donors and recipients as follows: (1) D+/R+; (2) D+/R-; (3) D-/R+; and (4) D-/R- in AMR and control group, where + means the presence of the variant while - means the reference nucleotide. After this analysis, only one SNP found in *adenosine deaminase* (*ADA*; p.Val178(p=), rs244076; **Figure 3A**), related to B-cell biology, had a statistically significant distribution (**Table 4**, P<0.02). Moreover, the presence of this allele had an inverse association with the appearance of AMR (D-/R- vs. D+/R+ or D+/R- or D-/R+), with an OR of 0.07 (95% CI: 0.01–0.44, P=0.006). This variant could modify the exonic splicing enhancer (**Table 5**), and thus, it could have an impact on protein expression.

Discussion

This study is the first analysis of the effect of donor and recipient-donor pair variants, in genes involved in the complement pathway and B-cell biology, related to AMR in HT patients. We identified 1 SNP, p.Ile75Val in *interleukin 4 receptor- α* (*IL4Ra*; rs1805010), in donors that correlated with the development of AMR. Moreover, in the analysis of recipient-donor discrepancies, we identified 1 SNP, p.Val178(p=) in *ADA* (rs244076) related with B-cell biology,

associated with the absence of AMR. No SNP, however, in complement pathway genes, either in donor or in recipient-donor pairs, was associated with AMR. Thus, the present data are in concordance with several studies that noted the importance of donor genotype in allograft outcome.^{14,19–20,31} In fact, Biggins et al had noted that *IL28B* and *DDX58* SNP that are favourable when present in the recipient, are unfavorable when present in the donor graft.¹⁸

Donor Variant p.Ile75Val in *IL4Rα*: Association With AMR

In recent years, the number of studies focused, not only on recipient, but also on donor genotype in genes related to complement and B-cell biology that could have an effect on transplant outcome, have increased (Table 1). Twelve out of 22 studies reviewed noted an influence of donor genotype in five different types of transplantation (kidney, n=5; liver, n=3; heart, n=2; lung, n=1; and hematopoietic stem cell, n=1) with different endpoints studied (acute rejection, n=7; graft survival and outcome, n=6; infection, n=3).^{14,17–19,21,22,27,28,30,31,34,35} The genes associated with transplantation outcomes were interleukins (*IL4*, *IL6*, *IL10*, and *IL28B*),^{18,19,28,30,31,34,39} different proteins of the complement cascade (*C3*, *C5*, *CD59*, and *MBL2*),^{14,17,21,22} and two genes related to the activation of B cells (*CCR5* and *DDX58*).^{18,27}

Surprisingly, despite the importance of the complement cascade and B-cell biology in AMR, no studies have been carried out on the genes in these two pathways in HT with AMR. In this sense, in the present patients, no SNP in the complement cascade genes was associated with AMR. But, although no SNP in the IL genes was associated with AMR, the presence of the variant p.Ile75Val in *IL4Rα*, which codes for the interleukin-4 receptor subunit α, in donors, was associated with AMR.

IL-4Rα mediated signaling on B cells drives the production of type 2 antibody isotypes IgG1 and IgE through STAT6 in response to IL4 stimulation.⁴⁰ The extracellular variant p.Ile75Val, or p.Ile50Val relative to the mature peptide, in *IL4Rα* identified in the present cohort and associated with AMR has been described in several studies on asthma, type 1 diabetes, hyper-IgE syndrome, and severe eczema.^{41–43} The p.Ile75Val polymorphism, named rs180510 on dbSNP, has been described as a gain-of-function mutation due to a sustained STAT6 phosphorylation.⁴⁴ This variant was associated with an increase in total serum IgE in atopic asthma, related to its active role in isotype class-switch recombination.⁴² Thus, to identify a

Table 4. Genotype Frequency in HT

	ADA-p.Val178(p=)	
	AMR (n=14)	Non-AMR (n=14)
D+/R+	0	1
D+/R–	2	3
D–/R+	0	6
D–/R–	12	4
P-value	0.013	

AMR, antibody-mediated rejection; D, donor; HT, heart transplantation; R, recipient.

possible role of *IL4Rα* p.Ile75Val in AMR, we can speculate, based on all these studies, that donors with the allele Val75, associated with gain of function, would present more IgE/IgG due to sustained STAT6 phosphorylation. This modification would in turn increase IL4 stimulation, thereby skewing towards the T-helper cell 2 response by increasing B-cell activity.⁴⁵ Moreover, the major production of IgG by B cells could increase the activation of the complement pathway, which could increase the risk of AMR.⁴⁶ More research is needed, however, to analyze this hypothesis and the role of donor p.Ile75Val variant in AMR.

Recipient-Donor Genotype Discrepancy and AMR

Despite the fact that several studies have noted that recipient-donor genotype discrepancy in genes related to complement activation and B-cell biology could affect transplant outcome, the results were inconclusive because of the low number of genes analyzed and the different outcomes studied (Table 1). The present study analyzed 112 genes, in a more ambitious strategy, and we focused on AMR due to the importance of complement cascade and B-cells in this entity. We did not find any SNP in complement genes related to AMR either in donor or in donor-recipient pairs, in concordance with previous studies that failed to find an association with acute rejection²⁰ or with graft outcomes.^{16,23,24,26} Thus, complement genes related to AMR seem to be important in recipients, and not in donors, as we have previously noted.¹⁰

In contrast, all of the studies carried out in genes related to B-cell biology in transplantation noted a relationship with various outcomes: acute rejection;^{28,30–32,34–36} graft

Table 5. In Silico Analysis of ESE and Silencer Motifs of the Variants in *C1QC* and *ADA*

ESE finder matrices for SRp40, SC35, SF2/ASF and SRp55					
Variant	Reference motif		Mutant motif		Variation
	Linked SR protein	Reference motif (value 0–100)	Linked SR protein	Reference motif (value 0–100)	
p.Val178(p=) <i>ADA</i>	SC35	AGCCATTG (75.8)	SC35	GGCCATTG (86.5)	+14.2%
ESE motifs from HSF					
Variant	Reference motif		Mutant motif		Variation
	Reference motif	Motif value (0–100)	Mutant motif	Motif value (0–100)	
		Reference sequence		Mutant sequence	
p.Val178(p=) <i>ADA</i>					
	9G8	GTAGCC (63.4)	9G8	GGTGGC (66.9)	New site Site broken –100A

DA, adenosine deaminase; ESE, exonic splicing enhancer; HSF, human splicing finder.

outcome;^{27,29,33,34} and infection.^{18,19} In the present study, after analyzing more than 316 SNP in 61 genes, we found an association between recipient-donor discrepancy in only 1 synonymous SNP, p.Val178(p=), in *ADA* related to B-cell biology. Due to the more frequent presence of the alternative allele in the donor-recipient pair in the control group, this polymorphism could have a protective effect against AMR. Although it is a synonymous variant, silent SNP can alter the final protein conformation.^{47,48} In the present study, on *in silico* analysis, p.Val178(p=) could modify the exonic splicing enhancer and possibly have an impact on protein expression. More research is needed, however, to verify this hypothesis.

Most of the studies conducted in B-cell genes in different organ transplantation found an association with IL genes or *tumor necrosis factor-α* (Table 1). Thus, this is the first description of a variant in *ADA* related to a transplantation outcome: AMR. *ADA* is a ubiquitously expressed metabolic enzyme that plays an integral role in numerous cellular processes and is encoded by *ADA*.⁴⁹ *ADA* is well known due to a severe combined immunodeficiency (ADA-SCID) in which there is dysfunction of both B and T lymphocytes with decreased production of Ig, resulting from mutations in the gene encoding *ADA*.⁴⁹ Moreover, polymorphisms in *ADA* also have been related to other pathologies such as autism, asthma, and rheumatoid arthritis.^{47,50,51} In the Sharma et al study, a significant association was found between the present identified variant, p.Val178(p=), and a poor response to rheumatoid arthritis.⁴⁷ Unfortunately they did not describe any mechanism by which the variant could affect the response.

Study Limitations

The main limitations of the present study were (1) the relatively small sample size because of the unicentric study design, the low incidence of AMR and the availability of donor samples; and (2) the modification over time in the routine diagnosis of AMR on EMB, which in the present center was not included in the routine protocol until 2013. The donor SNP and also the recipient-donor discrepancies in B-cell genes associated with AMR identified in the present study, could introduce a new approach into the treatment of AMR and could open up a new field of study.

Conclusions

Donor polymorphisms as well as recipient-donor discrepancies in B-cell biology genes, could have an important role in the development of AMR. No variants in donor or in donor-recipient pairs in the complement pathways, however, seems to have an impact in AMR.

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Disclosures

The authors declare no conflict of interest.

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Supplementary Files

Supplementary File 1

Table S1. Analyzed genes involved in the complement system and B-cell biology

Table S2. Characterization of anti-HLA antibodies, immunosuppression regimen and AMR treatment

Table S3. Donor and donor-recipient SNP in genes involved in the complement system and B-cell biology

Please find supplementary file(s);
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